NOTE TO THE FILE
December 12, 1997

Subject: BNF00047 - BXN with Bollgard Cotton

Keywords:
Cotton, *Gossypium hirsutum*, BXN™, nitrilase, *Klebsiella pneumoniae* subsp. *ozaenae*, cryIA(c), Bollgard, *Bacillus thuringiensis* subsp. *kurstaki*, kanamycin resistance gene, *kan*^{r}, aminoglycoside 3'-phosphotransferase II (APH(3')II), neomycin phosphotransferase II (nptII).

Background

BXN with Bollgard cotton contains the BXN gene encoding the nitrilase enzyme and a synthetic version of the *cry*IA(c) gene encoding a truncated version of the CryIA(c) protein. In a submission dated September 18, 1997, Calgene provided to FDA a summary of the safety and nutritional assessment it had conducted on its BXN with Bollgard. Calgene noted that the safety of the nitrilase enzyme was addressed in a previous consultation on BXN cotton (BNF0004, incorporated by reference) and therefore, is not addressed in this submission.

Intended Effect and Food/Feed Use

The intended technical effect of this genetic modification of cotton is to confer tolerance to the broadleaf herbicide Buctril® (which contains bromoxynil as the active ingredient) as well as to confer resistance to certain lepidopteran insect pests. Calgene stated that the applications and uses of BXN with Bollgard cotton are the same as conventional cotton - to provide fiber for textile use, refined oil for human consumption, and cottonseed meal for animal feed.

Molecular Alterations and Characterization

The BXN with Bollgard cotton lines contain 1) the BXN gene (encoding nitrilase) derived from *Klebsiella pneumoniae* subsp. *ozaenae* and under the control of the 35S promoter and the *tml* 3' terminator; 2) the *cry*IA(c) gene (codon-optimized for expression in plants) derived from *Bacillus thuringiensis* subsp. *kurstaki* and having the Mac promoter (*mas* and 35S promoter hybrid) and the *mas* 3' terminator; and 3) the *kan*^{r} gene from *E. coli* Tn5 with its associated 35S promoter and the *tml* 3' terminator. The genes and their regulatory sequences were inserted using an *Agrobacterium*-derived vector pCGN4084. Calgene noted that this vector contains the same DNA sequences as pBrx75 (which was used to generate BXN cotton that was the subject of BNF0004) with the only differences being the inclusion of a 3.7 kb Mac promoter/*cry*IA(c)/*mas*3' chimeric gene in pCGN4084 and the orientation of the 35S/BXN/*tml*3' chimeric gene relative to the *kan*^{r} gene.

Expressed Protein

Calgene stated that three new proteins are expressed in BXN with Bollgard cotton: the enzyme nitrilase encoded by the BXN gene, a truncated version of the CryIA(c) protein encoded by the *cry*IA(c) gene, and aminoglycoside 3'-phosphotransferase II (APH(3')II) encoded by the *kan*^{r} gene. Calgene provided concentrations of the three proteins in seed (2.5 ppm for CryIA(c), 0.25 ppm for nitrilase, and 2.5 ppm for APH(3')), meal (2.25 ppm for CryIA(c), 1.4 ppm for nitrilase and 13.5 ppm for APH(3')II) on a per fresh weight basis. Calgene did not detect any of the three proteins in oil (detection limit of 0.01 ppm). Calgene stated that protein levels were determined by Western blot analysis for four transformation events and that the amounts given represent estimated maximums and were consistent across events.

Calgene stated that the safety of both the nitrilase enzyme and APH(3')II have been addressed previously. The safety of the BXN gene-encoded nitrilase was the subject of a consultation (BNF0004) that was completed on April 5, 1995. APH(3')II is approved for use as a selectable marker in the development of transgenic cotton (21 CFR 173.170 and 573.130).

The safe use of pesticidal substances is under the regulatory purview of the Environmental Protection Agency. Therefore, although Calgene presented information pertaining to the CryIA(c) protein, we have not addressed the safe use of the pesticide.

Compositional Analysis

Endogenous toxicants

Gossypol and cyclopropenoid fatty acids (CPFA) are naturally present in cotton and are considered to be undesirable anti-nutritional compounds. Calgene measured gossypol and CPFA levels in five BXN with Bollgard transformation events (31707, 31803, 31807, 31808, and 42317) and found them to be consistent with levels found in four traditionally bred varieties (Coker 130, STLVA887, STV474, DPL51) and to levels given for cotton in the literature.

Nutrients

Calgene also performed compositional analysis of nutrients in cotton seed, refined oil, and cottonseed meal in the transgenic lines from the transgenic lines derived from the five transformation events, and in the four traditionally bred lines mentioned above. Parameters measured include proximate analysis (moisture, crude fat/oil, protein, and ash) of cottonseed meal; fiber analysis (crude fiber, acid detergent and neutral detergent fibers) of cotton seed; fatty acid composition of refined cottonseed oil; and amino acid profile of cottonseed meal. According to Calgene, no significant differences were observed for any of these parameters between the transgenic lines and the traditionally bred lines.

Conclusions

Calgene has concluded that its transgenic cotton lines are not materially different in terms of food safety and nutritional profile from cotton varieties currently on the market. At this time, based on Calgene's description of its data and analyses, the Agency considers Calgene's consultation on cotton lines from transformation events 31707, 31803, 31807, 31808, and 42317 to be complete.

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